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ACUTE ORAL TOXICITY IN THE RAT - ACUTE TOXIC CLASS METHOD

AUTHOR:

STUDY SPONSOR:

TEST FACILITY:

QUALITY ASSURANCE REPORT

This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress.

This report has been audited by , considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

	29 October 2004	Standard Test Method Compliance Audit
	06 June 2005	Test Material Preparation
	09 June 2005	Animal Preparation
	06 June 2005	Dosing
	07 June 2005	Assessment of Response
	07 June 2005	Necropsy
§	14 July 2005	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

DATE: 7 OCT 2005

GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106 as amended by SI 2004/0994)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 2004/9/EC and 2004/10/EC.

These international standards are acceptable to the Regulatory agencies of the following countries: Australia, Austria, Belgium, Canada, the Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Republic of Korea, Luxembourg, Mexico, The Netherlands, New Zealand, Norway, Poland, Portugal, Slovenia, South Africa, Spain, Sweden, Switzerland, Turkey, the United Kingdom, and the United States of America.

This report fully and accurately reflects the procedures used and data generated.

DATE: 6/10/05

CONTENTS

ACUTE ORAL TOXICITY IN THE RAT – ACUTE TOXIC CLASS METHOD

SUMMARY

Introduction. The study was performed to assess the acute oral toxicity of the test material following a single oral administration in the Sprague-Dawley CD strain rat. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 423 "Acute Oral Toxicity – Acute Toxic Class Method" (adopted 17 December 2001)
- Method B1 *tris* Acute Toxicity (Oral) of Commission Directive 2004/73/EC

Method. A group of three fasted females was treated with the test material at a dose level of 2000 mg/kg bodyweight. Based on the results from this dose level further groups of fasted females were treated at a dose level of 300 mg/kg bodyweight. Dosing was performed sequentially.

The test material was administered orally as a solution in distilled water. Clinical signs and bodyweight development were monitored during the study. All animals were subjected to gross necropsy.

Mortality. All animals treated at a dose level of 2000 mg/kg were found dead or killed *in extremis*. There were no deaths noted in animals treated at a dose level of 300 mg/kg.

Clinical Observations. Signs of systemic toxicity noted in two animals treated at a dose level of 2000 mg/kg were hunched posture, ataxia, lethargy, decreased respiratory rate, noisy respiration, dehydration and diuresis. There were no signs of systemic toxicity noted in animals treated at a concentration of 300 mg/kg.

Bodyweight. The surviving animals showed expected gains in bodyweight over the study period.

Necropsy. Abnormalities noted at necropsy of the animals that died during the study were abnormally red lungs, dark liver, dark kidneys and clear liquid present in the stomach. No abnormalities were noted at necropsy of the animal that was killed *in extremis* or at necropsy of animals that were killed at the end of the study.

Conclusion. The acute oral median lethal dose (LD_{50}) of the test material in the female Sprague-Dawley CD strain rat was approximately 500 mg/kg bodyweight (GHS Category 4 300 - 2000 mg/kg bodyweight).

ACUTE ORAL TOXICITY IN THE RAT
- ACUTE TOXIC CLASS METHOD

1. INTRODUCTION

The study was performed to assess the acute oral toxicity of the test material following a single oral administration in the Sprague-Dawley CD strain rat. The method was designed to meet the requirements of the following:

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The rat was selected for this study as it is a readily available rodent species, historically used in safety evaluation studies, and is acceptable to appropriate regulatory authorities. The oral route was selected as the most appropriate route of exposure and the results are believed to be of value in predicting the likely toxicity of the test material to man.

The study was performed between 25 May 2005 and 22 June 2005.

2. TEST MATERIAL AND EXPERIMENTAL PREPARATION

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	
Chemical name	:	
Description	:	white solid
Batch number	:	RS4-56
Date received	:	15 April 2005
Storage conditions	:	approximately 4°C in the dark

The integrity of supplied data relating to the identity, purity and stability of the test material is the responsibility of the Sponsor.

2.2 Preparation of Test Material

For the purpose of the study the test material was freshly prepared, as required, as a solution at the appropriate concentration in distilled water.

Determination by analysis of the concentration, homogeneity and stability of the test material preparations was not appropriate because it was not specified in the Study Plan and is not a requirement of the Test Guideline.

3. METHODS

3.1 Animals and Animal Husbandry

Female Sprague-Dawley CD (Crl: CD® (SD) IGS BR) strain rats were supplied by Charles River (UK) Ltd, Margate, Kent, UK. On receipt the animals were randomly allocated to cages. The animals were nulliparous and non-pregnant. After an acclimatisation period of at least five days the animals were selected at random and given a number unique within the study by indelible ink-marking on the tail and a number written on a cage card. At the start of the study the animals were eight to twelve weeks of age. The bodyweights fell within an interval of ± 20% of the mean initial bodyweight of the first treated group.

The animals were housed in groups of three in suspended solid-floor polypropylene cages furnished with woodflakes. With the exception of an overnight fast immediately before dosing and for approximately three to four hours after dosing, free access to mains drinking water and food (Certified Rat and Mouse Diet (Code 5LF2) supplied by BCM IPS Limited, London, UK) was allowed throughout the study. The diet, drinking water and bedding were routinely analysed and were considered not to contain any contaminants that would reasonably be expected to affect the purpose or integrity of the study.

The temperature and relative humidity were set to achieve limits of 19 to 25°C and 30 to 70% respectively. Any occasional deviations from these targets were considered not to have affected the purpose or integrity of the study. The rate of air exchange was at least fifteen changes per hour and the lighting was controlled by a time switch to give twelve hours continuous light (06:00 to 18:00) and twelve hours darkness.

The animals were provided with environmental enrichment items which were considered not to contain any contaminant of a level that might have affected the purpose or integrity of the study.

3.2 Procedure

Using all available information on the toxicity of the test material, 2000 mg/kg was chosen as the starting dose.

Groups of fasted animals were treated as follows:

Dose Level (mg/kg)	Concentration (mg/ml)	Dose Volume (ml/kg)	Number of Rats
			Female
2000	200	10	3
300	30	10	3
300	30	10	3

All animals were dosed once only by gavage, using a metal cannula attached to a graduated syringe. The volume administered to each animal was calculated according to the fasted bodyweight at the time of dosing. Treatment of animals was sequential. Sufficient time was allowed between each group and each dose level to confirm the survival of the previously dosed animals.

The animals were observed for deaths or overt signs of toxicity ½, 1, 2 and 4 hours after dosing and subsequently once daily for up to fourteen days.

Individual bodyweights were recorded prior to dosing and seven and fourteen days after treatment or at death.

At the end of the observation period the surviving animals were killed by cervical dislocation. All animals were subjected to gross pathological examination. This consisted of an external examination and opening of the abdominal and thoracic cavities for examination of major organs. The appearance of any macroscopic abnormalities was recorded. No tissues were retained.

The sequence of dosing may not always follow the Test Guideline as shown in the schematic diagram in Appendix 1. It is Company Policy to minimise the number of animals used on each study in accordance with UK Government Home Office guidelines. The sequence of testing does not affect the final classification of the test material.

3.3 Evaluation of Data

Data evaluations included the relationship, if any, between the exposure of the animal to the test material and the incidence and severity of all abnormalities including behavioural and clinical observations, gross lesions, bodyweight changes, mortality and any other toxicological effects.

Using the mortality data obtained, an estimate of the acute oral median lethal dose (LD_{50}) of the test material was made as shown in the schematic diagram in Appendix 1.

4. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safepharm archives for five years, after which instructions will be sought as to further retention or disposal.

5. RESULTS

5.1 Mortality Data

Individual mortality data are given in Table 1.

All animals treated at a dose level of 2000 mg/kg were found dead or killed *in extremis* during the day of dosing or one day after dosing.

There were no deaths noted in animals treated at a dose level of 300 mg/kg.

5.2 Clinical Observations

Individual clinical observations are given in Table 2 and Table 3.

Signs of systemic toxicity noted in two animals treated at a dose level of 2000 mg/kg were hunched posture, ataxia, lethargy, decreased respiratory rate, noisy respiration, dehydration and diuresis.

There were no signs of systemic toxicity noted in animals treated at a concentration of 300 mg/kg.

5.3 Bodyweight

Individual bodyweights and weekly bodyweight changes are given in Table 4 and Table 5.

The surviving animals showed expected gains in bodyweight over the study period.

5.4 Necropsy

Individual necropsy findings are given in Table 6 and Table 7.

Abnormalities noted at necropsy of the animals that died during the study were abnormally red lungs, dark liver, dark kidneys and clear liquid present in the stomach. No abnormalities were noted at necropsy of the animal that was killed *in extremis* or at necropsy of animals that were killed at the end of the study.

6. CONCLUSION

The acute oral median lethal dose (LD_{50}) of the test material in the female Sprague-Dawley CD strain rat was approximately 500 mg/kg bodyweight (GHS Category 4 300 - 2000 mg/kg bodyweight).

Table 1 Mortality Data

Dose Level mg/kg	Sex	Number of Animals Treated	Deaths During Day of Dosing (Hours)				Deaths During Period After Dosing (Days)								Deaths
			½	1	2	4	1	2	3	4	5	6	7	8-14	
2000	Female	3	0	0	1	0	2*	-	-	-	-	-	-	-	3/3
300	Female	3	0	0	0	0	0	0	0	0	0	0	0	0	0/3
	Female	3	0	0	0	0	0	0	0	0	0	0	0	0	0/3

* = Includes one animal killed *in extremis*
- = All animals dead

Table 2 Individual Clinical Observations - 2000 mg/kg

Dose Level mg/kg	Animal Number and Sex	Effects Noted After Dosing (Hours)				Effects Noted During Period After Dosing (Days)													
		½	1	2	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2000	1-0 Female	0	0	HA	H	X													
	1-1 Female	0	0	X															
	1-2 Female	0	0	HA	H	HLRd RnDh ADuX*													

0 = No signs of systemic toxicity
H = Hunched posture
A = Ataxia
Dh = Dehydration
Du = Diuresis
L = Lethargy
Rd = Decreased respiratory rate
Rl = Laboured respiration
X = Animal dead
X* = Animal killed *in extremis*

Table 3 Individual Clinical Observations - 300 mg/kg

Dose Level mg/kg	Animal Number and Sex	Effects Noted After Dosing (Hours)				Effects Noted During Period After Dosing (Days)													
		½	1	2	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
300	2-0 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2-1 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2-2 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3-0 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3-1 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3-2 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

0 = No signs of systemic toxicity

Table 4 Individual Bodyweights and Weekly Bodyweight Changes - 2000 mg/kg

Dose Level mg/kg	Animal Number and Sex	Bodyweight (g) at Day			Bodyweight (g) at Death	Bodyweight Gain (g) During Week	
		0	7	14		1	2
2000	1-0 Female	203	-	-	185	-	-
	1-1 Female	208	-	-	206	-	-
	1-2 Female	206	-	-	186	-	-

- = Animal dead

Table 5 Individual Bodyweights and Weekly Bodyweight Changes – 300 mg/kg

Dose Level mg/kg	Animal Number and Sex	Bodyweight (g) at Day			Bodyweight Gain (g) During Week	
		0	7	14	1	2
300	2-0 Female	244	273	294	29	21
	2-1 Female	219	243	248	24	5
	2-2 Female	214	244	254	30	10
	3-0 Female	224	247	260	23	13
	3-1 Female	221	242	255	21	13
	3-2 Female	212	238	247	26	9

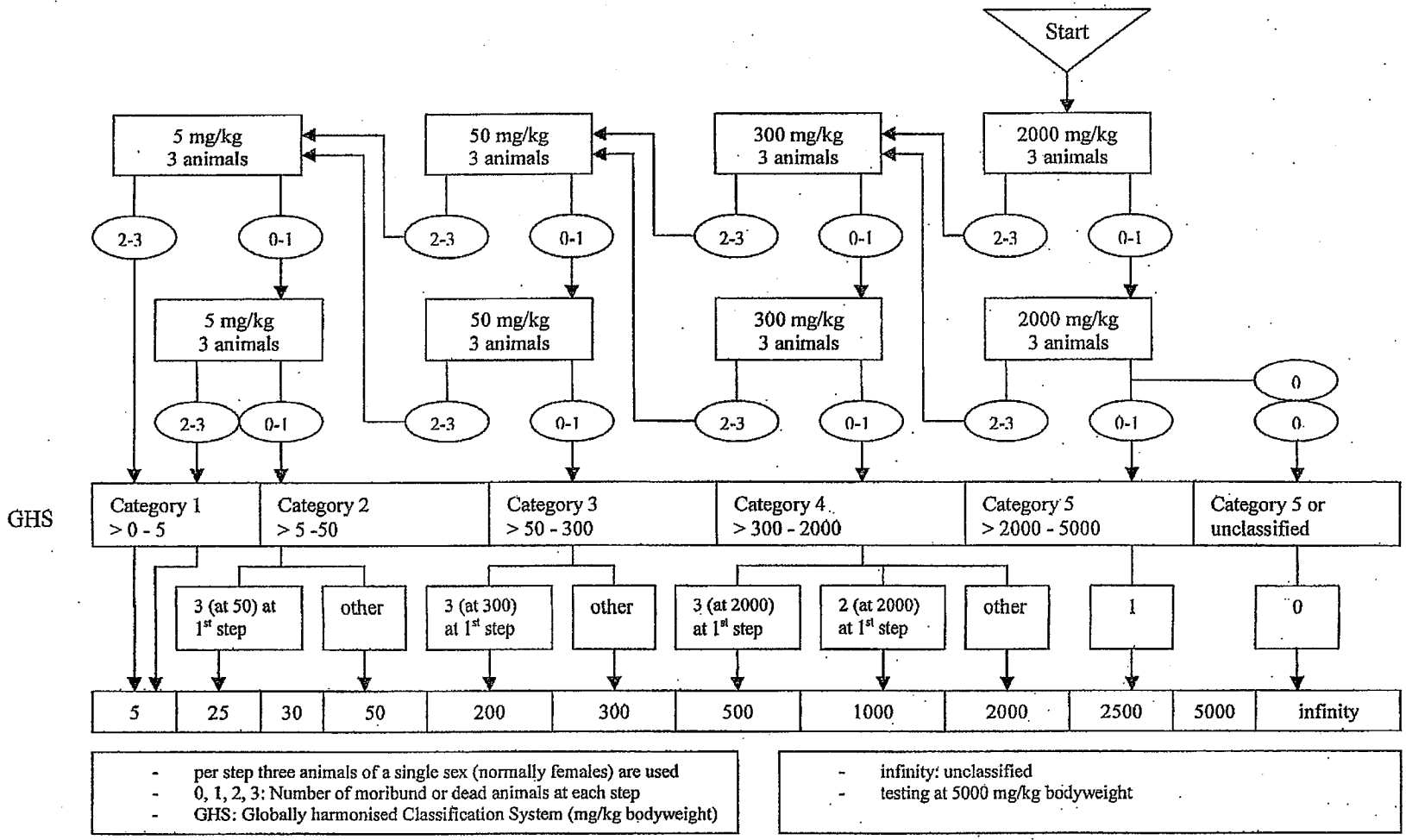
Table 6 Individual Necropsy Findings - 2000 mg/kg

Dose Level mg/kg	Animal Number and Sex	Time of Death	Macroscopic Observations
2000	1-0 Female	Found dead Day 1	Lungs: abnormally red Liver: dark Kidneys: dark
	1-1 Female	Found dead Day 0	Lungs: abnormally red Liver: dark Kidneys: dark Stomach: clear liquid present
	1-2 Female	Killed *in extremis* Day 1	No abnormalities detected

Table 7 Individual Necropsy Findings - 300 mg/kg

Dose Level mg/kg	Animal Number and Sex	Time of Death	Macroscopic Observations
300	2-0 Female	Killed Day 14	No abnormalities detected
	2-1 Female	Killed Day 14	No abnormalities detected
	2-2 Female	Killed Day 14	No abnormalities detected
	3-0 Female	Killed Day 14	No abnormalities detected
	3-1 Female	Killed Day 14	No abnormalities detected
	3-2 Female	Killed Day 14	No abnormalities detected

Appendix 1 Test Procedure with a Starting Dose of 2000 mg/kg Bodyweight



Appendix 2 Statement of GLP Compliance in Accordance with Directive 88/320/EEC

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

DATE OF INSPECTION

2nd December 2002

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

Roger G. Alexander
13/2/03

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority

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I verify that this is an exact copy of the original report which is located in the Archives of

DATE: 14/10/05